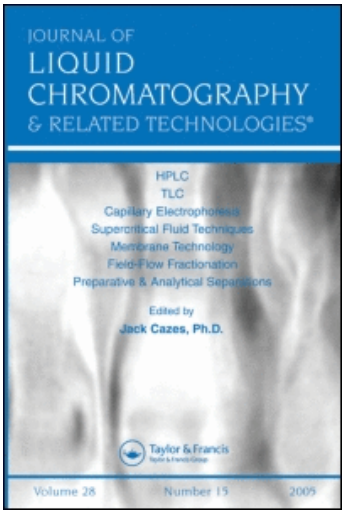


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# Determination of Matrine and Oxymatrine in *Sophora Flavescens Ait*. via High Performance Liquid Chromatography

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**Abstract:** In the present work, a reversed phase high performance liquid chromatographic method (HPLC) has been developed and validated for analysis of matrine and oxymatrine in *Sophora Flavescens Ait*. HPLC separation of the alkaloids was performed on a $C_{18}$ column. A mobile phase composed of methanol-water-trifluoroacetic in a ratio of 16:84:0.002 (v/v) was found to be the most suitable mobile phase for this separation. The extracted amounts are 0.0091 and 0.15 mg/g and the relative standard deviations were 3.3% for the matrine and 2.9% for the oxymatrine analysis.

**Keywords:** Matrine, Oxymatrine, RP-HPLC, *Sophora flavescens Ait.*

## INTRODUCTION

*Sophora flavescens Ait*. (SFA), designated in Sheng Nong's Herbal Classic as a moderate medicine, is cold by nature with a bitter taste, entering three channels, the heart, spleen, and kidney. According to Herbal Classic of Hundreds of Medicinal Materials, the dried roots

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of SFA are used to treat hotness in the heart with bitter entering heart and cold removing internal hotness. With the development of separation and extraction techniques, intensive investigations and studies have been conducted in China on SFA. Notably, it has been found that matrine type alkaloids are present in SFA.[1] Matrine type alkaloids can obviously inhibit a variety of clinical gastric mucosa damage. This may result from the direct neutralization of external hydrochloric acid and gastric acid by matrine. In addition, the mitigation of matrine has certain protective effects on gastric mucosa.[2]

By means of inhibiting enterocinesia, matrine type alkaloids have visible antidiarrheal effects.[3] They also can kill amoebas and giardia lamblia stiles.[4] Matrine can kill trichomonads and slow down the development of subcutaneous abscesses caused by parasites, and also can cure infections caused by mouse vaginal trichomoniasis. It has also been found that matrine can potentially be applied as a medicine for killing parasites, but the mechanism is not clear at the present.[5] It has been held that this kind of structure might be the group accounting for the positive myotome effects in matrine type alkaloids, which may be related to the activation of the calcium channel.[6]



Due to the high pharmacological activities of matrine type alkaloids in SFA, the herb has recently attracted attention in natural medication research. Several papers have been published concerning the separation and quantification of the alkaloids in SFA, and several methods such as high performance liquid chromatography (HPLC),[7,8] high performance capillary electrophoresis (HPCE),[9,10] and gas chromatography (GC)[11] have been applied to the separation and determination of matrine type alkaloids in the SFA root. HPLC is the most widely used separation technique for this application owing to its simplicity and general applicability to matrine type alkaloids.[12,13] HPCE techniques can be used when HPLC is not suitable or efficient for the samples of interest. Sample preparation is the most important aspect in the application of HPLC or HPCE. Extraction procedures including liquid–liquid extraction, solid-phase extraction, and other methods, can be selected according to requirements of precision, accuracy, and reproducibility.[14–16]

Following a detailed study, this report describes a simple, sensitive, and more reliable HPLC method for simultaneous determination of two bioactive alkaloids, matrine and oxymatrine, in SFA, using ultraviolet detection and isocratic elution. The analysis time was 25 min per injection. The suggested method has been successfully applied to the determination of matrine and oxymatrine in dried roots.

## EXPERIMENTAL

### Chemicals

Standards of matrine and oxymatrine (analytical grade) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). SFA was purchased from a local market. Methanol (HPLC Grade) was purchased from Duksan Pure Chemical. Co., Ltd. (Korea). Trifluoroacetic acid (TFA) was purchased from Acros Organics (USA). Water was twice distilled and filtered (FH-0.45 μm, Advantec MFS, Inc., Japan) using a decompression pump (Division of Millipore, Waters, USA).

### HPLC Analysis



The chromatography system consisted of a Waters 600 s Multi Solvent Delivery System and a Waters 616 liquid chromatography system (Waters Associates, Milford, MA, U.S.A.), a Rheodyne injector (Cotati, CA, USA) valve with a 20 μL sample loop, and a variable wavelength 2487 UV dual channel detector. Millennium software (Ver. 3.2 Interface Eng., Korea) on a PC was used as a data acquisition system. Experiments were performed with a commercially available RStech Corporation $C_{18}$ bonded phase column (4.6×150 mm i.d. 100 Å pore sizes, and 5 μm particles) purchased from Rs-Tech Co. (Daejeon, Korea). A mobile phase composed of methanol-water-trifluoroacetic in a ratio of 16:84:0.002 (v/v) was found to be the most suitable mobile phase for this separation, and enabled baseline separation of the two analytes free from interference with isocratic elution. The injection volume was 5 μL, the flow rate was 0.5 mL/min, and the UV wavelength was set at 210 nm.

### Preparation of Solutions

The stock standard solution was prepared by transferring approximately 1 mg of matrine and oxymatrine reference standards, respectively, each accurately weighed to a 5 mL volumetric flask, and, respectively, adding about 1 mL of water and methanol to the flask. These standard stock solutions were stored and further diluted to different working standard solutions at 4°C in a refrigerator in darkness.

The SFA roots were oven dried, sliced, and crushed into powder for the extraction experiments. Selection of a solvent affects the efficiency of extraction. Water, methanol, ethanol, ethyl acetate, and chloroform were selected as extraction solvents.

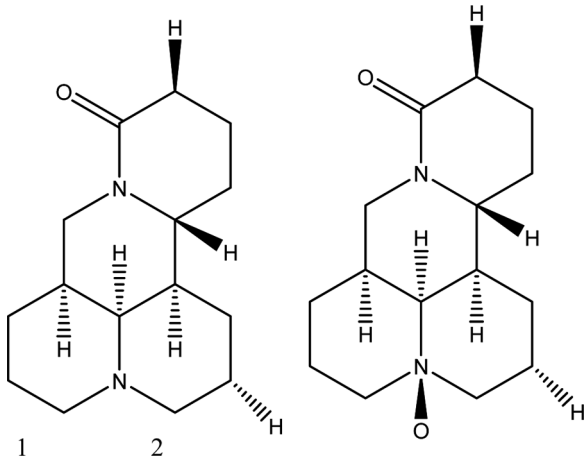


***Figure 1.*** Structural formulae of two alkaloids.



## RESULTS AND DISCUSSION

The composition of SFA is very complicated. This herb is known to contain approximately 10 alkaloids, matrine and oxymatrine being the main bioactive alkaloids. The structures of the two alkaloids are very similar (Figure 1), thus, the results of the separation and analysis were often unsatisfactory when other alkaloids were simultaneously determined.

### Effect of Different Extraction Solvents

The different extraction solvents used in the experiment for the extraction of matrine and oxymatrine from SFA were water, methanol, ethanol, ethyl acetate, and chloroform. Each solvent of 50 mL was used to extract 0.5 g of SFA powder during a period of 12 hr under room temperature, respectively. Table 1 shows that both two compounds could be extracted by polar solvents and the highest extracted amount was obtained with water.

### Effects of Different Extraction Methods

The different extraction methods such as dipping extraction, and ultrasonic extraction were investigated by extracting 0.5 g of SFA powder extracted with 50 mL of water. In dipping extraction, the SFA powder was mixed and stirred with the solvent for different times. In Figure 2, it is seen that the extracted amounts of matrine and oxymatrine increased as the dipping times was increased from 0.5 hr to 9 hr, and there was no

*Table 1.* Extracted amounts of matrine and oxymatrine with different solvents

| Compounds Solvents | Matrine (mg/g) | Oxymatrine (mg/g) |
|---|---|---|
| Water | 0.0085 | 0.15 |
| Methanol | 0.0066 | 0.12 |
| Ethanol | 0.0009 | 0.005 |
| Ethyl acetate | * | * |
| Chloroform | * | * |

*Not detected.

obvious increase after further prolonged extraction time. Equivalent samples were then prepared by an ultrasonic method without dipping time. Figure 3 shows that the amounts of extracted matrine and oxymatrine increased with an increase of ultrasonic time. However, comparing the results of the two methods, it was found that the amounts extracted via the ultrasonic method were lower, while more energy was required in the experiments. Thus, it was determined that the ultrasonic method is not appropriate for this approach.



## Optimum Extraction Temperature

Different dipping temperatures ranging from 30°C to 100°C were evaluated while the dipping time was fixed at 4 hr. The results are shown in

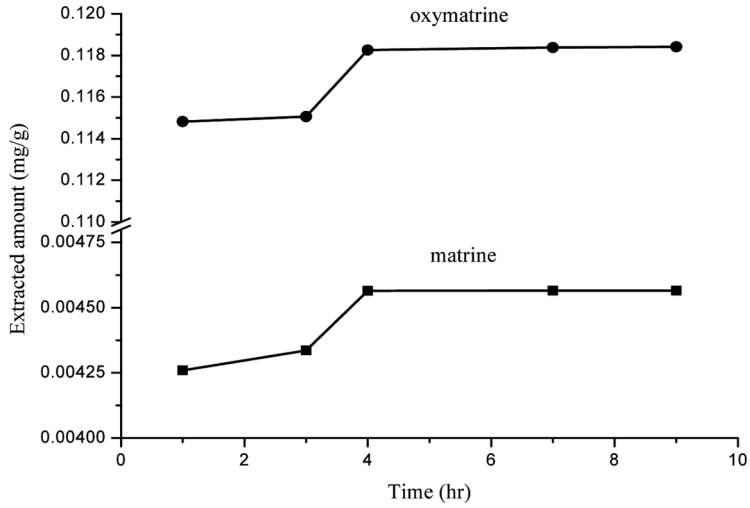


*Figure 2.* Effect of different dipping times on extracted amounts of SFA.

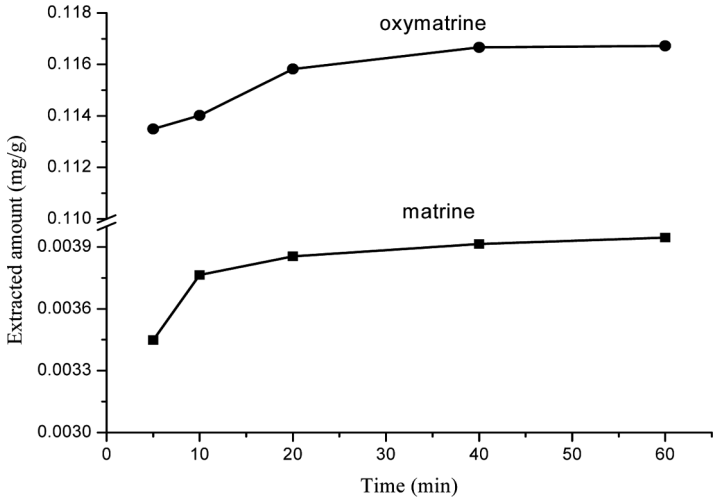


***Figure 3.*** Effect of different ultrasonic times on extracted amounts of SFA.



Figure 4. The amounts of extracted matrine and oxymatrine increased quickly with an increase of temperature increasing from 30°C to 80°C, and were fairly constant beyond 80°C. Comparing the results with those obtained via the dipping method, the amounts of matrine and oxymatrine extracted from SFA via 4 hr dipping under room temperature were lower than those for 4 hr dipping under 80°C. An extraction time of 4 hr under

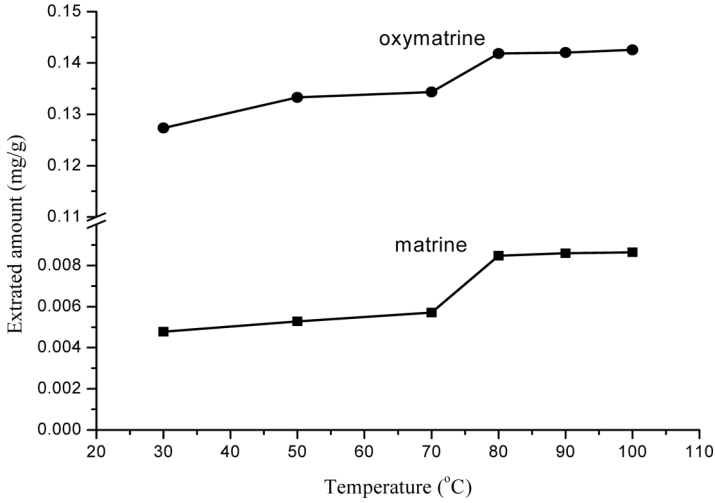


***Figure 4.*** Effect of different temperatures on extracted amounts of SFA.

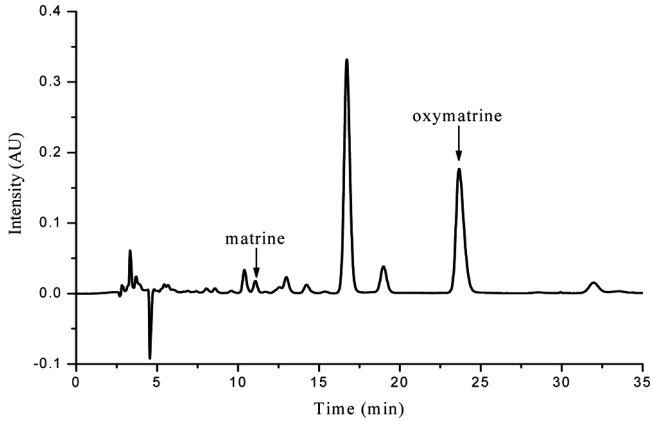


***Figure 5.*** Chromatogram of matrine and oxymatrine in water extract.



a temperature of 80°C is the optimum condition for the extraction of matrine and oxymatrine from SFA.

## Chromatographic Separation

Figure 5 shows typical chromatograms of SFA samples. The use of water as an extract solvent and an extraction time of 4 hr under a temperature of 80°C are the optimum conditions for the extraction of matrine and oxymatrine from SFA. Under the chromatographic conditions described above, matrine and oxymatrine had retention times of approximately 11.2 and 24.8 min, respectively. The analysis time was 25 min per injection. It can be seen from the figure that good separation and detectability of matrine and oxymatrine in the SFA sample were obtained with baseline resolved peaks and chromatograms with minimal interference from the herb. Hence, it is relatively easy to estimate the peak area with acceptable accuracy.

## Stability of the Solutions

The stability of standard and sample solutions was determined by monitoring the peak area and migration time of standard mixture solutions and sample solutions over a period of 1 week. The results showed that the migration time and peak area of each analyte remained almost unchanged, and that no significant degradation is observed within

***Table 2.*** Calibration curve for the quantification of matrine and oxymatrine

| Analyte | Calibration curve ($r^2$) | Test range (μg/mL) |
|---|---|---|
| Matrine | $Y = 17073.0X + 4879.9$ (0.9999) | 3.0–500.0 |
| Oxymatrine | $Y = 15333.0X + 59432.0$ (0.9991) | 3.0–1000.0 |

$Y$, peak area; $X$, concentration of analyte (μg/mL).

the given period, indicating the solutions are stable for at least 1 week without the results being affected.

## Linearity

A series of samples containing matrine (3.0, 10.0, 50.0, 100, 250.0, and 500.0 μg/mL) and oxymatrine (3.0, 10.0, 50.0, 100.0, 500.0, and 1000.0 μg/mL) were prepared to study the relationships between the peak area and the concentrations of the alkaloids under selected conditions. The results showed that the peak area was linearly related to the amount of matrine for a range of 3.0–500.0 μg/mL. For oxymatrine, the linear range was 3.0–1000.0 μg/mL. The results of these linearity studies are presented in Table 2.



## Precision

In order to determine the accuracy of the method recovery studies were carried out. Known amounts of the two alkaloids were added to an accurately weighed fine powder sample of SFA. A mixture of the alkaloids was extracted and analyzed using the proposed method. The control solution was prepared by extracting the same two samples without adding the alkaloids.

***Table 3.*** Recovery studies of matrine and oxymatrine in SFA (n = 3)

| Analyte | Amount added (μg/mL) | Average recovery (%) | RSD (%) |
|---|---|---|---|
| Matrine | 5.0 | 90.3 | 3.3 |
| | 10.0 | 90.9 | 3.1 |
| | 50.0 | 91.1 | 2.9 |
| Oxymatrine | 50.0 | 88.5 | 2.9 |
| | 100.0 | 87.6 | 2.8 |
| | 500.0 | 88.3 | 2.8 |

The mean recoveries of matrine and oxymatrine from SFA were evaluated by spiking three different levels of matrine (3.0, 10.0, 50.0 μg/mL) and oxymatrine (30.0, 100.0, 500.0 μg/mL) to the sample in replicates of three. The measured concentrations were compared with the theoretical concentrations to calculate the recovery rates. The experimental results showed that the average recoveries of the two alkaloids were 90.8% for matrine and 88.1% for oxymatrine (Table 3). Comparison with the real sample analysis verified that the values noted above were of acceptable precision and accuracy.

## CONCLUSION

The proposed method provides excellent separation and good precision, and is simple and reliable for both chromatographic conditions and sample preparation. Furthermore, the analytical procedure is relatively straightforward and is very suitable for the determination of alkaloids in SFA. The use of water as an extract solvent and an extraction time of 4 hr under a temperature of 80°C are the optimum conditions for the extraction of matrine and oxymatrine from SFA. The extracted amounts of matrine and oxymatrine are 0.0091 and 0.15 mg/g and the recoveries are 90.8% and 88.1% with relative standard deviations of 3.3% and 2.9%, respectively. The method has been successfully applied to the simultaneous determination of matrine and oxymatrine in SFA.



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